A PHARMACEUTICAL COMPOSITION FOR TREATING RHEUMATISM AND THE PREPARATION THEREOF

THE FIELD OF THE INVENTION

The invention is directed to a medicine for treating rheumatism, and the medicine's preparation.

THE BACKGROUND OF THE INVENTION

It is believed that the rheumatoid and rheumatoid arthritis (RA) is refractory and about 18,000,000 RA patients have been disabled because of this disease. The medicine research for curing RA has continued about a century. Aspirin is the first medicine which is widely used to treat RA. The medicine to treat RA can be divided into 2 kinds: non-steroidal anti-inflammatory drugs (NSAIDs) and immunosuppressive agents. NSAIDs includes cyclophthasine, antinfan and adrenal cortex hormone. The clinical researchs have proven the effectiveness of NSAIDs. The immuneosuppressive agents include methotrexate, cyclophosphane, penicillamine etc. Immunoregulation has become one of the important therapies in the recent years. But all the medicines which are used to treat rheumatism have serious side-effects. The medicine which can treat rheumatism effectively with low toxicity hasn't been invented before the present invention.

There are 3 directions in the research of antirheumatics that should be emphasized. The first direction is NSAIDs and cytokine-antagon, such as recombined soluble TNFαntagon, IL-1 inhibitor and PAF inhibitor. The second direction is the new immunosuppressive agent and

immunomodulator, such as cyclosporin A. The third direction is the compound medicines.

In traditional Chinese medicine (TCM), the research on the "Bi Zheng" (equal to the definition of rheumatism in the modern medicine) can be traced back to the Han dynasty more than 1,500 years ago. Three prescriptions: "Ma Xing Shi Gan decoction", "Fangji Huangqi decoction" and "Wutou decoction", which were used to treat "Bi Zheng" were recorded in the medicine classics "Shanghan Lun" written by the famous doctor Zhang Zhongjing at that time. A kind of wild plant "huo ba hua"(Gelsemium elegans Bentll) in the Sichuang province has been proven effective to treat rheumatism in a clinical research carried out in the local area(Sichuang province). But the further study found that it had a serious side-effect on the reproductive organs and some other uncontrollable problems.

The treatment of "arthralgia disease" by the methods of TCM has reached a high level after development by numerous doctors in such a long history. By now, there are many effective prescriptions and herbs. There are more than 80 kinds of herbs and 29 kinds of patent medicines recorded in the China pharmacopoeia 1995 edition and 2000 edition. But there are still many problems, for example: ① the effect is not good enough in treating the serious arthralgia diseases such as rheumatoid arthritis; ② the dosage forms can not meet the demands of modern life. ③ some medicine has good effects, but the side-effects are too serious, such as the extract of *triperygium wilfordii*. So, it is necessary to develop new antirheumatic medicines that are highly-effective, create minimal noxiousness, and are convenient for administration. This medicine

should have the similar effects and lower side-effects than synthetic artificial anti-rheumatic medicine.

SUMMARY OF THE INVENTION

The invention provides an antirheumatic that is highly-effective, creates minimal noxiousness, is convenient for administration, and its preparation thereof.

The medicine uses the following crude herbs:

Tripterygium hypoglaucum (Levl.) Hutch;

Epimedium brevicornum Maxim;

Lycium barbarum L; and,

Cuscuta chinensis Lam (or *Cuscuta australis* R. Br.)

DETAILED DESCRIPTION OF EXEMPLARY EMBODIMENTS

The antirheumatic medicine of the present invention utilizes the crude herbs as follows:

Tripterygium hypoglaucum (Levl.) Hutch;

Epimedium brevicornum Maxim;

Lycium barbarum L; and,

Cuscuta chinensis Lam (or *Cuscuta australis* R. Br.)

The crude herbs to produce the antirheumatic medicine can be combined in several ways. The *tripterygium hypoglaucum* (Levl.) Hutch is the necessary herb, one or two or three of the other three herbs can be added to make the compound prescription.

One of the optimal crude herbs rate of the compound prescription is as following:

Tripterygium hypoglaucum (Levl.) Hutch. 1-4 parts by weight

Epimedium brevicornum Maxim. 1-4 parts by weight
Lycium barbarum L. 1-4 parts by weight
Cuscuta chinensis Lam. 1-4 parts by weight

The other optimal crude herbs rate of the material is as following:

Tripterygium hypoglaucum (Levl.) Hutch. 2 parts by weight
Epimedium brevicornum Maxim. 2 parts by weight
Lycium barbarum L. 1 parts by weight
Cuscuta chinensis Lam. 1 parts by weight

The third optimal crude herbs rate of the material is as following:

Tripterygium hypoglaucum (Levl.) Hutch. 1-4 parts by weight
Epimedium brevicornum Maxim. 1-4 parts by weight

The fourth optimal crude herbs rate of the material is as following:

Tripterygium hypoglaucum (Levl.) Hutch. 2 parts by weight
Epimedium brevicornum Maxim. 2 parts by weight

The fifth optimal crude herbs rate of the material is as following:

Tripterygium hypoglaucum (Levl.) Hutch 1-4 parts by weight
Epimedium brevicornum Maxim 1-4 parts by weight
Lycium barbarum L 1-4 parts by weight

The sixth optimal crude herbs rate of the material is as following:

Tripterygium hypoglaucum (Levl.) Hutch 2 parts by weight
Epimedium brevicornum Maxim 2 parts by weight
Lycium barbarum L 1 parts by weight

The seventh optimal crude herbs rate of the material is as following:

Tripterygium hypoglaucum (Levl.) Hutch 1-4 parts by weight
Epimedium brevicornum Maxim 1-4 parts by weight
Cuscuta chinensis Lam 1-4 parts by weight

The eighth optimal crude herbs rate of the material is as following:

Tripterygium hypoglaucum (Levl.) Hutch 2 parts by weight

Epimedium brevicornum Maxim 2 parts by weight

Cuscuta chinensis Lam 1 parts by weight

The content of the icariin ($C_{33}H_{40}O_{15}$) in the medicine combinations above should not be less than 2.0 mg.

The optimal crude herbs rate of the material can be the other way as following:

Tripterygium hypoglaucum (Levl.) Hutch 1-4 parts by weight

Lycium barbarum L 1-4 parts by weight

And / or *Cuscuta chinensis* Lam 1-4 parts by weight

The optimal crude herbs rate of the material can be another way as following:

Tripterygium hypoglaucum (Levl.) Hutch 2 parts by weight

Lycium barbarum L 1 part by weight

And / or *Cuscuta chinensis* Lam 1 part by weight

The crude herbs are prepared on the rate and then they can be made into any dosage forms used in the clinic, such as the bolus form, the powder forms, the ointment forms, the tablet forms, the soft or hard capsule forms, the granule forms, the injection forms and so on.

The preparation method of the invented medicine is as following:

The crude herbs are prepared on the weight rate:

Tripterygium hypoglaucum (Levl.) Hutch 1-4 parts by weight

Epimedium brevicornum Maxim 1-4 parts by weight

Lycium barbarum L 1-4 parts by weight

Cuscuta chinensis Lam 1-4 parts by weight

The *Tripterygium hypoglaucum* (Levl.) Hutch. and *Epimedium*

brevicornum Maxim are cut into pieces. Then the pieces are decocted by water for 2 ~ 4 times separately. The *Lycium barbarum* L and *Cuscuta chinensis* Lam are soaked in the hot water (80~95℃) for 1 ~ 3 times separately. The decoction fluid and the immersion fluid of the herbs are collected and added to the corresponding macroscopic void adsorbent resin column separately. After the adsorption, the columns are washed with water until the flushing liquor turns clear. Then the resins are eluted by 60%-80% alcohol. The eluting liquors are collected from the time when its color turns deep till the color turns very weak. Then the alcohol in the column is pushed out by high pressure water and mixed with the eluting liquor. The total mixed eluting liquor is about 3 ~ 8 times concentrated of the correspondent crude herb. All the 4 eluting liquors are recovered from the alcohol, and condensed to their specific density of 1.10 separately. The condensed liquors are dried by a spray drying method to get the extract of the crude herbs. The 4 kinds of extracts are mixed uniformly to be made into any dosage form that are needed by the clinic.

The optimal preparation method of the invented medicine is as follows:

The crude herbs are prepared on the weight rate:

Tripterygium hypoglaucum (Levl.) Hutch	2 parts by weight
Epimedium brevicornum Maxim	2 parts by weight
Lycium barbarum L	1 part by weight
Cuscuta chinensis Lam	1 part by weight

The *Tripterygium hypoglaucum* (Levl.) Hutch. is cut into pieces. Then the pieces are added with 13, 10, 10 times weight of the water to

decoct 3 times respectively. Each time is for 1 hour. The *Epimedium brevicornum* Maxim is cut into pieces. Then the pieces are added with 15, 10, 10 times weight of the water to decoct 3 times respectively. Each time is for 1 hour. The *Lycium barbarum* L is smashed to coarse powder and soaked in the hot water (80℃, 20 times weight of the crude herb) 3 times. Each time is for 1 hour. The *Cuscuta chinensis* Lam is smashed to coarse powder and soaked in the hot water (80℃, 31 times weight of the crude herb) 3 times. Each time is for 1 hour. The decoction fluid and the immersion fluid of the herbs are filtrated separately and added to the correspondent macroscopic void adsorbent resin column(the type of the resin is JD-1 (WLD resin)) (manufactured by the Chinese Traditional Medicine Institute of Sichuan Province). Another useful resin is D_{101}, (manufactured by Nankai University Resin Factory, Tianjin). After the adsorption, the resins in the columns are eluted by 70% alcohol. The eluting liquors are collected from when its color turnins deep till the color turns very weak. The alcohol is recovered from the eluting liquor. Then the rest of the liquor is condensed and dried to get the extract powder. The 4 kinds of extract powders are mixed uniformly to be made into any dosage forms that are needed by the clinic.

The invented medicine can be prepared by the method as follows:

The crude herbs are prepared on the weight rate which is recorded before. The *Tripterygium hypoglaucum* (Levl.) Hutch. and *Epimedium brevicornum* Maxim are cut into pieces. The *Lycium barbarum* L and *Cuscuta chinensis* Lam are crushed or not. The 4 kinds of herbs are extracted in 0~95% alcohol **at 10~98℃** for 1~4 times separately or together. The extracted liquors are mixed or not. Then the extracted

liquors are recovered from the alcohol, then condensed, dried, smashed and mixed uniformly or proportionally. The mixed powder can be made into any dosage form needed in the clinic.

The invented medicine can be made from the effective constituents of the 4 herbs.

The effective constituents of *Epimedium brevicornum* Maxim are icariin, icariside I , icariside II, and Icariin A. The effective constituents of *Tripterygium hypoglaucum* (Levl.) Hutch. are diterpenes, triterpenes and alkaloids compounds. The effective constituents of *Lycium barbarum* L and *Cuscuta chinensis* Lam are both flavones.

The crude herb *Epimedium brevicornum* Maxim can be replaced by one or more kinds of the effective constituents of itself, such as icariin, icariside I , icariside II, and Icariin A. The crude herb *Tripterygium hypoglaucum* (Levl.) Hutch. can be replaced by one or more kinds of the effective constituents of itself, such as diterpenes, triterpenes and alkaloids compounds. While the *Lycium barbarum* L and *Cuscuta chinensis* Lam can be replaced by flavones.

It has been proven by pharmacodynamics research that the invented medicine (Fengshiping Capsule) could inhibit the primary and secondary injury adjuvant arthritis (AA). It could inhibit the delayed hypersensitivity (DTH) in the ear of a mouse caused by the 2,4 dinitrofluorobenzene (DNFB). It could inhibit the antibody production of the hemolysin and the activity of the IL-1, IL-2, IL-6 and TNF in the macrophage and splenocyte. The Fengshiping Capsule could inhibit the lymphocyte transformation induced by the ConA. It could inhibit the CD_4、CD_8 cells remarkably, especially CD_4 cells, but it can not affect

the rate of CD_4/CD_8 very much. There was a remarkable linear relationship between the dosage and the effect. 12~18g (crude medicine)/kg was the minimum effective dose. The invented medicine could inhibit the activity of the NK cells. In the effective dose, the Fengshiping Capsule did not cause the atrophy of the important immune organs such as thymus and spleen, and did not inhibit the phagocytic activity of the macrophage.

The invented medicine had a remarkable anti-inflammatory action. It could inhibit the over penetrating condition of the capillary in the mouse's abdominal cavity caused by the injection of acetic acid. It could inhibit the swelling in the ear of the mouse caused by the croton oil. It could inhibit the pleuritis in the mouse and the assembling of the WBC to the CMC cyst in the rat induced by the carrageenan. But the invented medicine couldn't obviously inhibit the rat's foot swelling induced by the carrageenan and the granuloma caused by the tampon. The Fengshipng Capsule could remarkably inhibit the body-twist reaction caused by the acetic acid in the mouse.

Experimental example 1: the effect on the adjuvant arthritis (AA)

1.1 The preventing effect on the AA of the invented medicine

72 isogenous SD rats of the same batch, half male and half female, 180 ~ 220g weight each, were divided randomly into 6 groups. Each group has 12 rats. Each 6 rats lived in a cage. The perimeter of the double ankle joints and the feet of the rats were measured accurately and recorded as the normal value. All the rats were given the same volume of the invented medicine in different concentrations of the solution of

tragacanth orally. 1 hour later, all the rats were injected with 0.1ml Freund's complete adjuvant (FCA) under the skin of the left postpedes. In the next 30 days, all the rats were given orally the correspondent medicine once a day at the same dosage. And on these days, the perimeters of the double ankle joints and the feet of the rats were measured once a day. In this experiment, the swelling degree ($\triangle$cm) equals the difference value of the perimeters measured after the FCA injection and before the FCA injection. (See the result in table 1.1 and 1.2) At the end of the experiment, the major organs of the rats were weighted. (See the table 1.3, 1.4)

Table 1.1 The effect of the Fengshiping on the swelling degree of the left ankle joint and foot after the injection of FCA in the rat AA model ($\overline{X} \pm S$)

Group	Dose (g/kg)	Swelling degree (△cm)						
		1d	2d	3d	9d	12d	14d	16d
Control	-	0.69±0.17	0.69±0.12	0.92±0.18	0.84±0.41	1.10±0.30	1.65±0.68	2.10±0.55
Fengshiping	7.5	0.74±0.12	0.66±0.074	0.83±0.13	0.77±0.27	1.11±0.45	1.34±0.53	1.91±0.61
Fengshiping	15	0.80±0.24	0.62±0.13	0.76±0.18	0.49±0.17*	0.73±0.34*	1.00±0.48*	1.38±0.67*
Fengshiping	30	0.75±0.19	0.67±0.19	0.87±0.28	0.63±0.22	0.73±0.34*	0.82±0.43**	1.05±0.53**
Tripterygium hypoglaucum (Levl.) Hutch.	5	0.72±0.11	0.68±0.16	0.91±0.18	0.66±0.23	0.88±0.29	1.03±0.36*	1.37±0.33*
prednisone	0.01	0.64±0.14	0.64±0.16	0.50±0.26	0.46±0.25	0.72±0.46*	0.87±0.46**	1.28±0.69*

Group	Dose (g/kg)	Swelling degree(△cm)					
		18d	20d	22d	24d	26d	28d
Control	-	2.18±0.44	2.05±0.46	2.00±0.46	2.04±0.57	1.92±0.65	1.83±0.67
Fengshiping	7.5	1.74±0.73	1.81±0.55	1.81±0.52	1.77±0.55	1.65±0.55	1.55±0.49
Fengshiping	15	1.32±0.59**	1.28±0.58**	1.34±0.61*	1.33±0.67*	1.20±0.64*	1.08±0.58**
Fengshiping	30	0.95±0.50**	0.87±0.51**	0.95±0.54**	0.89±0.59**	0.90±0.57**	0.86±0.51**
Tripterygium hypoglaucum (Levl.) Hutch.	5	1.47±0.43**	1.50±0.43**	1.49±0.43*	1.42±0.53*	1.40±0.56*	1.32±0.57
prednisone	0.01	1.18±0.7**6	1.03±0.67**	1.05±0.69*	0.90±0.64**	0.86±0.65**	0.85±0.59**

Comparing to the control group *P<0.05 , **P<0.01(the signs have the same meaning in the following tables)

1.2 The effect of the Fengshiping on the swelling degree of the left ankle joint and foot after the injection of FCA in the rat AA model ($\overline{X} \pm S$)

Group	Dose (g/kg)	Swelling degree (△cm)					
		2d	9d	12d	14d	16d	18d
Control	-	0.14±0.05	0.06±0.10	0.34±0.36	0.80±0.52	1.43±0.67	1.36±0.61
Fengshiping	7.5	0.18±0.06	0.10±0.14	0.26±0.36	0.82±0.52	1.31±0.64	1.28±0.71
Fengshiping	15	0.15±0.08	0.02±0.06	0.13±0.10*	0.37±0.31*	0.90±0.56*	0.79±0.60*
Fengshiping	30	0.18±0.09	0.06±0.06	0.16±0.08*	0.29±0.20**	0.49±0.41**	0.33±0.29**
Tripterygium hypoglaucum (Levl.) Hutch.	5	0.16±0.07	0.01±0.07	0.11±0.10	0.44±0.19**	0.87±0.56*	0.84±0.67*
prednisone	0.01	0.20±0.06	0.08±0.08	0.21±0.16	0.44±0.43	0.99±0.63	0.84±0.74*

Group	Dose (g/kg)	Swelling degree (△cm)				
		20d	22d	24d	26d	28d
Control	-	1.28±0.57	1.38±0.64	1.35±0.75	1.20±0.78	1.12±0.63
Fengshiping	7.5	1.33±0.71	1.31±0.73	1.27±0.73	1.16±0.73	1.07±0.65

Fengshiping	15	1.74±0.57*	1.92±0.61*	0.95±0.64*	0.88±0.58*	1.83±0.55
Fengshiping	30	0.27±0.30**	0.34±0.31**	0.32±0.33**	0.31±0.32**	0.34±0.32**
Tripterygium hypoglaucum (Levl.) Hutch.	5	0.82±0.65*	0.89±0.70*	0.80±0.67*	0.83±0.68	0.75±0.69
prednisone	0.01	0.82±0.72*	0.79±0.74*	0.75±0.67**	0.68±0.64*	0.71±0.67

1.3 The effect of the Fengshipng on the body weight of the AA rats ($\overline{X} \pm S$)

Group	Dose (g/kg)	Body weight change(g)		
		Initiative BW	BW at 1 month later	BW change
Control	-	228±34	231±52	3
Fengshiping	7.5	229±34	220±46	-9
Fengshiping	15	223±40	232±34	9
Fengshiping	30	224±37	256±60	32
Tripterygium hypoglaucum (Levl.) Hutch.	5	226±45	230±43	4
prednisone	0.01	264±55	244±31	-21

1.4 The effect of the Fengshiping on the organ weight of the immune system in the AA rats (prevention experiment)($\overline{X} \pm S$)

Group	Dose (g/kg)	Organ index [(organ weight/body weight)/100]			
		Liver	Spleen	Thymus	adrenal gland
Control	-	3.92±0.65	0.34±0.10	0.098±0.040	0.027±0.01
Fengshiping	7.5	3.73±0.29	0.31±0.09	0.078±0.038	0.027±0.008
Fengshiping	15	3.48±0.32	0.38±0.10	0.100±0.034	0.023±0.005
Fengshiping	30	3.38±0.28*	0.44±0.12*	0.100±0.032	0.022±0.007
Tripterygium hypoglaucum (Levl.) Hutch.	5	3.21±0.30**	0.36±0.05	0.052±0.011**	0.026±0.009
prednisone	0.01	3.04±0.20**	0.32±0.08	0.050±0.060**	0.020±0.004*

1.2 The therapeutic effect on the AA of the invented medicine

50 male SD rats were divided into 5 groups at random. The model building was the same as the prevention experiment, but the correspondent medicines were given orally 13 days after the injection of the FCA. The medicines were given once a day for 2 weeks. The swelling degree (△cm) was the difference of the perimeters between the value of first administration day and the other days. (See the result in

table 1.5, 1.6) The major organs' weight is showed in table 1.7.

1.5 The therapeutic effect of Fengshiping on the swelling degree of The left anklejoint and foot in the AA rats ($\overline{X} \pm S$)

Group	Dose (g/kg)	Swelling degree (△cm)			
		1d	2d	4d	6d
Control	-	1.81±0.27	1.92±0.19	2.12±0.22	2.16±0.27
Fengshiping	7.5	1.68±0.50	1.64±0.54	1.70±0.57	1.82±0.61
Fengshiping	15	1.44±0.41*	1.51±0.36**	1.65±0.34**	1.74±0.31**
Fengshiping	30	1.50±0.56	1.48±0.41**	1.51±0.44**	1.59±0.51**
prednisone	0.01	1.78±0.51	1.70±0.51	1.63±0.50*	1.58±0.50**

Group	Dose (g/kg)	Swelling degree (△cm)			
		8d	10d	12d	14d
Control	-	1.92±0.32	1.87±0.34	1.92±0.39	1.78±0.44
Fengshiping	7.5	1.67±0.68	1.60±0.71	1.61±0.77	1.58±0.71
Fengshiping	15	1.46±0.37**	1.48±0.30*	1.28±0.37**	1.22±0.38**
Fengshiping	30	1.29±0.58**	1.29±0.65**	1.26±0.67**	1.20±0.68*
prednisone	0.01	1.27±0.46**	1.09±0.54**	0.94±0.50**	0.94±0.42**

1.6 The therapeutic effect of Fengshiping on the swelling degree of the right anklejoint and foot in the AA rats ($\overline{X} \pm S$)

Group	Dose (g/kg)	Swelling degree (△cm)			
		2d	4d	6d	8d
Control	-	0.36±0.26	0.45±0.25	0.55±0.34	0.47±0.29
Fengshiping	7.5	0.12±0.25	0.34±0.32	0.48±0.41	0.28±0.38
Fengshiping	15	0.21±0.18	0.38±0.27	0.44±0.33	0.21±0.33*
Fengshiping	30	0.10±0.48	0.06±0.28**	0.11±0.24**	0.06±0.27**
prednisone	0.01	0.10±0.13*	0.15±0.28*	0.11±0.25**	-0.08±0.34**

Group	Dose (g/kg)	Swelling degree(△cm)		
		10d	12d	14d

Control	-	0.48±0.25	0.46±0.31	0.40±0.36
Fengshiping	7.5	0.35±0.30	0.30±0.29	0.30±0.35
Fengshiping	15	0.19±0.45*	0.06±0.31**	-0.06±0.34**
Fengshiping	30	0.02±0.39**	0.05±0.38*	-0.02±0.41**
prednisone	0.01	-0.13±0.28**	-0.26±0.36**	-0.33±0.39**

n = 10 , comparing with the control group , *P<0.05 , **P<0.01

1.7 The effect of the Fengshiping on the organ weight of the immune system in the AA rats ($\overline{X} \pm S$)

Group	Dose (g/kg)	Organ index [(organ weight/body weight)/100]			
		Liver	Spleen	Thymus	adrenal gland
Control	-	0.35±0.23	0.35±0.061	0.073±0.014	0.026±0.0071
Fengshiping	7.5	3.21±0.52	0.33±0.091	0.071±0.026	0.024±0.0085
Fengshiping	15	3.40±0.54	0.36±0.014	0.067±0.022	0.023±0.0048
Fengshiping	30	2.79±0.43	0.32±0.083	0.069±0.029	0.023±0.0072
Tripterygium hypoglaucum (Levl.) Hutch.	5	3.92±0.59	0.35±0.100	0.075±0.034	0.027±0.0060
prednisone	0.01	3.52±0.35	0.28±0.047*	0.05±0.011**	0.02±0.0043*

The data shown in the tables 1.1, 1.2, 1.3, 1.5 and 1.6 proves that the Fengshiping could strongly inhibit the primary and secondary injury caused by FCA, whenever the medicine was given at the beginning of the FCA injection or 2 weeks after the FCA injection. The experiments prove that the Fengshiping has both the preventing and the therapeutic effect. By comparing the effect of Fengshiping on the swelling degree in the anklejoint and foot, we found that the Fengshiping could inhibit the specific immuno-swelling in the ankle joint better than the nonspecific immuno-swelling in the foot of rats. This result indicates that the main effect of Fengshiping was inhibiting the immunity inflammatory

reaction.

The data in the tables 1.3, 1.4 and 1.7 show that the AA rats had no obvious BW increase during the period of the experiment. In the group given the Fengshiping with the effective dosage, the rats still had BW increase. In the groups of prednisone and preventing, the BW of rats had decreased, while the thymus and adrenal gland were atrophied. In the group of *tripterygium hypoglaucum* (Levl.) Hutch, the thymus had not atrophied yet. But in the 3 groups given the different dosage of Fengshiping, no atrophy of the thymus and adrenal gland were observed.

1.3 The pathologic change of the AA after the treatment of the invented medicine in rats

45 SD rats, 180±20g weight each, were divided into 6 groups. After the AA caused by FCA appeared, all the rats were given orally, Fengshiping solution for 5 days once a day. 1 hour after the last administration, the joint index of the rats was evaluated and calculated. The secondary injuried postpedes' joints on the opposite of the FCA injectiont were taken off and soaked in the formaldehyde. After the tissues were HE tinted, the pathological change of the synovium and cartilage were observed and recorded. The data are shown in table 1.8.

1.8 The effect of Fengshiping on the AA joint index in the rats $(\overline{X} \pm S)$

Group	Dose (g/kg)	Rat number	Joint index
Control	-	8	0**
AA model	-	7	6.2±0.49
Fengshiping	7.5	9	4.86±0.90**
Fengshiping	15	7	4.71±0.95**

Fengshiping	30	7	4.56±1.13**
Glucosidorum Tripterygll Totorum	0.006	7	4.57±0.79**

Comparing with the model groupP<0.01**

The joint index was the sum of the inflammatory scores of the four limbs. According to the degree of inflammatory, each limb was evaluated on the criteria as following: normal (0), red without swelling (1), red and swelling (2), seriously swelling (3), deforming and stiffness (4).

Observed from the microscope, the joint synovial membranes of the rat posterior limb were hyperplasia in the model group; the collagen fiber had increased; and there was infiltration of lymphocytes and plasma cells in the tissue. An obvious granuloma had formed. The synovial cells had degenerated and the cytochylema had been tinted red; the caryon had been pycnosis; the epithelium had exfoliated in some part of the synovial membrane. The cartilage became atrophied; the surface of it was rough and some of the chondrocytes had proliferated lightly.

After the treatment with the Fengshiping, the inflammation of the joint synovial membrane was inhibited, more collogen fiber was produced; less synovial cells exfoliated ; the cells on the surface of the cartilage had proliferated and the surface had turned smooth. The cartilage was in a recovering condition.

Experimental example 2: The effect of Fengshiping on the delayed hypersensitivity reaction (DTH) caused by 2,4-DNFB in the ear of the mouse

50 NIH mice, half male and half female, were divided into 5 groups. Each mouse was led into a hypersensitivity reaction by using the 1%

DNFB acetone solution at a dosage of 0.025ml at the right place of the abdomen where the fur had been removed. Using the same solution on the same place enhanced the hypersensitivity reaction on the third day. On the fifth day, all the mice were smeared with the 1% DNFB edible oil solution at the mice's right ears at a dosage of 0.01 ml each. 24 hours later, all the mice were killed. The mouse' s 2 ears were weighed by the torsion balance and the difference of the 2 ears was recorded as the DTH degree caused by the DNFB. The experiment was carried out on the different immune and administration processes.

2.1 The effect on the DTH by the full course administration

The immune and administration processes is as follows:

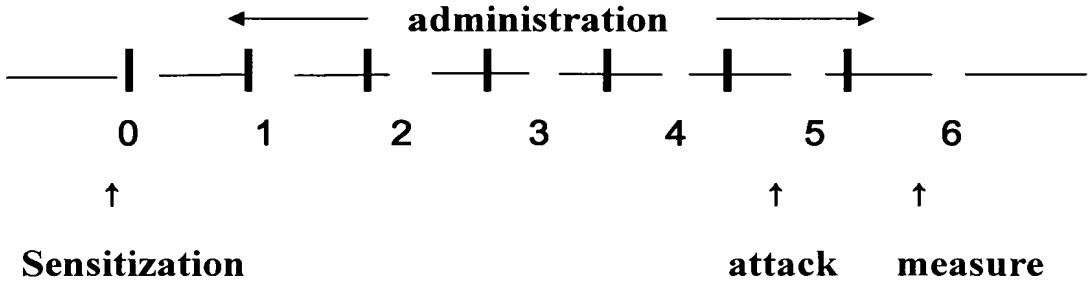

Table 2.1 The effect of Fengshiping on the DTH caused by DNFB in the NIH mouse ($\overline{X} \pm S$)

group	dose (g/kg)	Administration time (day)	Mice number	Percent of ear swelling	Percent of inhibition (%)	P value
control			10	34.20±3.77		
Fengshiping	27	0~5	10	26.24±3.34	23.3	<0.01
Fengshiping	40	0~5	10	12.99±4.96	62.0	<0.01
Fengshiping	60	0~5	10	10.43±7.53	69.5	<0.01
cortisumman	0.003	0~5	10	13.93±4.41	59.3	<0.01
control			10	42.43±5.28		
Fengshiping	40	-2~0	10	31.50±10.52	25.0	<0.01
Fengshiping	40	-2~2	10	30.88±7.92	27.2	<0.01
Fengshiping	40	-2~5	10	21.07±4.62*	50.3	<0.01
Fengshiping	40	5~6	10	32.00±9.37	41.7	<0.01
cyclophosphane	0.05	-2~2	10	39.40±10.78	8.1	>0.05
cyclophosphane	0.05	-2~0	10	37.47±6.71	11.7	>0.05
control			10	38.50±4.67		
cyclophosphane	0.1×3	0、2、4 day once a day	10	23.00±7.65	40.3	<0.01
cyclophosphane	0.25	-3d	10	41.84±7.75	-8.7	
Fengshiping	60	0~4	10	27.20±10.20	29.4	<0.01
cyclophosphane +Fengshiping	0.25 + 60	-3,0~4	10	38.07±6.65	1.1	

*comparing with the other groups P<0.05 或 P<0.01

According to the data shown in table 2.1, it indicated that the Fengshiping had an obvious inhibiting effect on the DTH caused by DNFB. There was a significant relationship between the dosage and the effect. The inhibiting activity increased when the dosage increased. The inhibiting percent could reach 69.5% on the dosage of 60.9g/kg.

2.2 The effect on the DTH of the different administration time

The immune and administration processes and the correspondingt results have been shown in the middle and bottom parts of table 2.1. According to the results showed in the middle part of the table 2.1, all the administration ways could significantly inhibit the DTH of the mouse in spite of the administration beginning from the 2 days before the sensitization to ending at the sensitization, or beginning from the 2 days before the sensitization to ending 2 days after the sensitization, or beginning from the 2 days before the sensitization to ending 5 days after the sensitization, or beginning before the attack and ending after the attack. But the administration way that began 2 days before the sensitization and ended 5 days after the sensitization had the most powerful inhibiting activity. It indicated that the Fengshiping could inhibit the DTH by a compound mechanism that it could inhibit the cells participating in the early period of the DTH, the effector cells in the advanced period and the cells related to the DTH in the middle period. This mechanism was different from that of the cyclophosphane. On a small dosage, the cyclophosphane didn't affect the DTH, if its administration method began from the 2 days before the sensitization and ended at the sensitization day or 2 days after the sensitization day.

Based on the bottom part of the table 2.1, if a high dosage of

cyclophosphane was given to the mouse at one time 3 days before the sensitization, the function of the Th cells would turn sthenic because of the powerful inhibition on the Ts cells. The DTH in the mouse would be enhanced. If the cyclophosphane was used with the Fengshiping in this administration method, it could lower the inhibiting activity of Fengshiping. This result indicated that the Fengshiping has a different machnism to the cyclophosphane in the control of DTH. The Fengshiping may have a higher activity in inhibiting the THcells.

Experimental example 3: The effect on the humoral immunity

3.1 The effect on the product of the hemolysin antibody caused by the chick RBC

190 mice, 18-22g weight, half male and half female, were divided into 19 groups. Each mouse was immunized with 5% CRBC solution 0.2 ml. The Fengshiping solutions were given orally to the mice at the different times. 7 days after the immunization, all the mice were sampled using the blood from the eyes. Then the blood samples were diluted and the level of the hemolysin antibody was measured. The results are shown in table 3.1, 3.2 and 3.3.

Table 3.1 The effect of Fengshiping on the produce of the hemolysin antibody in the NIH mouse ($\overline{X} \pm S$)

group	dose (g/kg)	Administration time	Mouse number	Hemolysin value	Inhibiting percent (%)	P value
control			10	169.0±62.0		
Fengshiping	18	0~7	10	46.0±15.6	72.8	<0.01
Fengshiping	27	0~7	10	35.4±12.0	79.1	<0.01
Fengshiping	40	0~7	10	28.2±5.9	83.3	<0.01

Fengshiping		60	0~7	10	16.7±3.0	90.1	<0.01
Tripterygium hypoglaucum Hutch.	(Levl.)	13.3	0~7	10	121.0± 88.0**	28.4	<0.015
cyclophosphane		0.02	0~7	10	35.0±12.0	79.3	<0.01

** comparing with the Fengshiping (40g/kg) group P<0.01

Table 3.2 The effect of Fengshiping on the produce of the hemolysin antibody in the ICR mouse ($\overline{X} \pm S$)

group		dose (g/kg)	Administration time	Mouse number	Hemolysin value	Inhibiting percent (%)	P value
control		-	-	10	124.70±42.60		
Fengshiping		12	0~7	10	75.00±53.10	39.9	<0.05
Fengshiping		18	0~7	10	45.60±22.70	63.4	<0.01
Fengshiping		27	0~7	10	29.10±22.10	76.8	<0.01
Fengshiping		40	0~7	10	28.20±5.30	77.4	<0.01
Tripterygium hypoglaucum Hutch.	(Levl.)	6.0	0~7	10	143.50±67.90**		>0.05
cyclophosphane		0.02	0~7	10	27.80±6.60	77.9	<0.01

**comparing with the Fengshiping (18g/kg) group P<0.01

Table 3.3 The effect of Fengshiping on the produce of the hemolysin antibody in the ICR mouse ($\overline{X} \pm S$)

group	dose (g/kg)	Administration time	Mouse number	Hemolysin value	Inhibiting percent (%)	P value
control	-	-	10	256.0±26.0		
Fengshiping	18	-7~7	10	198.0±50.0	22.7	<0.01
Fengshiping	18	-3~7	10	156.0±85.0	39.1	<0.01
Fengshiping	18	0~7	10	98.0±35.0	61.7	<0.01
cyclophosphane	0.02	0~7	10	25.0±4.0	90.2	<0.01

According to the data in table 3, the Fengshiping has a remarkable inhibiting effect on the product of the hemolysin antibody in the different mouse species and this effect would increase along with an

increase of the dosage. There was a certain relationship between the dosage and the effect. The lowest effective dosage was 12g/kg. Compared with the same quantity of Tripterygium hypoglaucum (Levl.) Hutch, the Fengshiping had a higher inhibiting activity. Based on the data in table 3.1, the inhibiting activity of Fengshiping was 2.25 times higher than the Tripterygium hypoglaucum (Levl.) Hutch. The inhibiting activity of Tripterygium hypoglaucum (Levl.) Hutch. with the dosage of 13.5g/kg was weaker than that of the Fengshiping which contains 6g/kg Tripterygium hypoglaucum (Levl.) Hutch).

3.2 The effect of the Fengshiping on the humoral immunity in the AA mouse

The NIH mice, 20±2g weight, were injected with 0.05 ml FCA under the vola skin of the right postpedes. 3 weeks later the AA model mouse builded. The model mice were divided into 6 groups randomly and given orally the corresponding medicines for 5 days. At the beginning of the administration, all the mice were sensitized with 0.5ml 10% sheep RBC (SRBC). Five days later, all the mice were killed. Their spleens were taken out and washed by the Hank's liquor to prepare the lymphocyte suspended liquor. The concentration of the cells was adjusted to 2×10^7 / ml. 1 ml lymphocyte suspension, 1 ml 0.2% SRBC and 1 ml 1:30 addiment were added to one test tube. The tube was put in the water bath at 37℃ for 1 hour. Then the tube was centrifugated at 2000rpm for 5 minutes. The supernatant fluid was separated and tested its sptical density at the 415nm wavelength on the 722 type apeotrophotometer. The value was representative of PFC quantity.

The other share of the blood samples from the sensitized mice was

separated the serum to test the potency of the antibody. The measured data were recorded on the way of Log2 value. (See the data in table 3.4)

Table 3.4 The effect of Fengshiping on the humoral immunity in the mouse ($\overline{X} \pm S$)

group	dose (g/kg)	Mouse number	PFC (OD)	IgM(Log2)
control	-	8	0.819±0.013#	6.875±0.641
AA model group	-	10	0.940±0.019**	7.700±0.599*
fengshiping	5	8	0.834±0.012**#	6.875±0.641#
fengshiping	10	8	0.834±0.012**#	6.750±0.886#
fengshiping	20	8	0.830±0.014**#	6.375±0.518##
Glucosidorum Tripterygll Totorum	0.012	10	0.835±0.015**#	6.950±0.597#

Comparing with the control group *P<0.05，**P<0.01；comparing with the model group # P<0.05，## P<0.01

According to the table 3.4, the levels of PFC and IgM in the AA mouse were higher than that of the normal mouse. The Fengshiping could lower the value of the PFC and IgM in the AA mouse significantly.

Experimental example 4: The effect of the Fengshiping on the passive cutis anaphylactic reaction (PCA) in the rat.

The rats were injected with the egg albumn at 10mg/kg in the muscle. At the same time, all the rats were injected with 2×10^{10}(0.2ml) bordetella pertussis in the abdominal cavity for sensitization. 2 weeks later, all the rats were killed to sample the blood. All the blood samples were separated for preparing the serum.

60 rats, 150~200g, half male and half female, were divided into 6

groups at random. In the light narcosis condition induced by ether, each rat was shaved on its back and injected with the 2 concentrations of anti-egg-album serum 0.1ml under the skin at the shaved place. The serums were diluted to the concentrations of 1:5(d1) and 1:10(d2) before the experiment. 48 hours later, all the rats were intravenously injected with the 0.5% Evans blue normal saline solution 1 ml which contains 1 mg egg albumin. 20 minutes later, the rats were killed by decapitation. The rats' back skins were dissected and turned over. According to the dark and light and areas of the blue stains exudated from the vessels, all the rats were evaluated by several people. The skins stained by the Evans blue were scissored and soaked in 5ml 0.1% sodium sulfate acetone (7:3) solution for 48 hours. Then it was centrifuged to separate the supernatant liquor. The optical density of the supernates was measured at the wavelength 590nm to calculate the degree of the PCA reaction and the inhibiting percent. The results were shown in table 4.

Table 4 The effect of Fengshiping on the PCA in rat ($\overline{X} \pm S$)

Group	dose (g/kg)	value		absorbancy	
		d_1	d_2	d_1	d_2
Control	-	5.60±1.78	2.40±2.46	0.191±0.129	0.096±0.106
Fengshiping	12	7.50±2.51	4.20±2.49	0.402±0.213*	0.192±0.175
Fengshiping	24	7.10±2.13	4.10±1.79	0.310±0.177	0.137±0.099
Fengshiping	48	6.00±1.83	1.70±1.95	0.121±0.109	0.024±0.026*
Tripterygium hypoglaucum (Levl.) Hutch.	8	6.11±1.27	2.56±1.67	0.223±0.122	0.074±0.045
Ketotifen	0.1	2.78±1.64**	0.67±1.41	0.033±0.024**	0.027±0.019*

Comparing with the control group *P<0.05 , **P<0.01

According to the table 4, it is indicated that the Fengshiping had a weak effect on the PCA in the rat. Only at a high dosage, the inhibiting

effect of Fengshiping was obviously different from that of the control group.

Experimental example 5: The effect of Fengshiping on the cytokines.

5.1 The effect of Fengshiping on the levels of TNFα and IL-2 in the mouse.

60 ICR mice, 18~22g, half male and half female, were divided into 6 groups at random. Each group was given orally the corresponding medicines including the different dosages of Fengshiping and the other medicines. The medicines were administrated once a day for 10 days. 24 hours after the last administration, samples from the mice were taken, including the macrophage and spleen cells from the abdominal cavity in the aseptic condition. The samples were washed with Hank's liquor twice and non-serum RPIM 1640 liquor once. Then the washed samples were diluted to the suspension with the 5% FCS-RPMI 1640 at the concentration of 2×10^8 / ml. Then the suspensions were added with 10ng/ml LPS or the 10ng/ml ConA and cultured in the 5% CO2 condition for 48 hours at 37℃. Then the cultured suspensions' TNF α and IL-2 levels were measured using the usual methods.

The measurement of TNFα

The plate was coated by mouse TNF-α monoclonal antibody. The plate had cultured supernate added at the dose of 50μl/ hole. Then the plate was kept still for 60 minutes at the room temperature. Then the plate was mixed with biotin antibody marker at 25℃ for 2 hours. Then the enzyme labeled avidin was added into the plate and left for 30 minutes. After adding the substrate constant for 30 minutes, the stop

liquor was added to the plate. The mixed liquor was measured using the OD value at the wavelength of 450nm. The content of the TNF-α(ng/ml) was calculated on the data of OD value by the method of standard curve.

The measurement of the IL-2:

The CTLL cells which were on the logarithmic growth phase and whose growth depends on the IL-2, were adjusted to the suspension at the concentration of 1×10^5/ml with the 5% FCS-RPMI 1640. Then the 96 hole cell culturing plate was filled with the CTLL cell suspension to the quantity of 100μl/hole. The supernates were added to the quantity of 100μl/hole and each sample was added to 3 holes. The samples cultured were compared with the different dilutions of standard rHIL-2 and the control sample (culture fluid) to measure the IL-2. All the samples were cultured in the 5% CO2 for 24 hours at 37℃. 6 hours before the end of the culture, all the samples were centrifuged and separated from the supernate. Each hole had 110 μl of supernate removed and then 10 μl of MTT was added. The samples were cultured for 3 hours at 37℃, and then the OD was measured at the wavelengths of 570nm and 630nm. The final OD value of the sample was the difference of OD (570nm) and OD (630nm).

$$\text{IL-2 activity} = \frac{\text{Sample}\overline{\text{OD}} - \text{Control (Culture Fluid)}\overline{\text{OD}}}{\text{Standard Sample}\ \overline{\text{OD}} - \text{Control (Culture Fluid)}\overline{\text{OD}}} \times \text{activity of the standard sample (IU/ml)}$$

Table 5.1 The effect of Fengshiping on the TNF αnd IL-2 ($\overline{X} \pm S$)

group	dose (g/kg)	Mouse number	TNF (pg/ml)	IL-2 (IU/ml)
Control	-	10	87.80±14.63	26.30±4.22
Fengshiping	12	10	62.14±13.13**	16.00±2.89**
	24	10	58.60±9.63**	18.80±2.86**
	36	10	54.40±10.88**	18.20±2.86**
Tripterygium hypoglaucum (Levl.) Hutch.	8	10	58.25±10.32**	16.00±2.88**
cyclophosphane	0.02	10	42.20±9.57**	10.10±3.00**

*P<0.05 , **P<0.01

According to the data in Table 5.1, it suggested that the Fengshiping have a obvious inhibiting effect on the TNFα. On the dosage of 12g/kg, the medicine had showed an obvious inhibiting effect. Along with the increase of the dosage, the inhibiting effect increased. But the dosage-effect curve went evenly. The Fengshiping had an obvious inhibiting effect on the IL-2, but no dosage-effect relationship was observed.

5.2 The effect of Fengshiping on the IL-1, IL-6

70 NIH mice, 18-22g weight, half male and half female, were divided into 7 groups at random. All the groups were given orally the corresponding medicines (fengshiping and the other medicines). The medicines were given once a day for 10 days. 24 hours after the last administration, all the mice were killed and the macrophage and spleen cells from the abdominal carvity were sampled. The IL-1 and IL-6 in the samples were measured.

The measurement of IL-1:

The macrophages in the abdominal cavity were sampled in the asepsis condition. Then the samples were washed by the Hank's liquor 2

times and nonserum RPMI1640 liquor 1 time. Then the clear samples were adjusted to the 4×10^6 / ml cell suspension with 5% FCS-RPMI liquor. 1 ml of the suspension was added to the test tube and cultured at 37℃ for 1 hour. The unadherent cells were abandoned. Then the cultured liquor was added with 5% FCS-RPMI 1640 and LPS (10ng/ml) to culture. The cells were cultured in 5% CO_2 at 37℃ for 72 hours. During the course, the cultured cells were freezed and thawed several times. The final product was saved at 4℃. The C57 mices' thymuses in the asepsis condition were sampled. Then the samples were prepared to the 1×10^6/ml cell suspension with 5% FCS-RPMI1640.

100μl supernate separated from the frost thawing liquor and 100μl cell suspension of thymus were added into the 96-hole flat bottom cell-culture plate. Each sample was cultured in 3 holes and compared with the different dilutions standard rHIL-1 and the control sample (culture fluid). Each hole had 2ng ConA added and then the plate was cultured in the 5% CO_2 at 37℃ for 72 hours. 14 hours before the end of the culture, each hole had 3H-TdR 0.1μCi added. The cultured cells were collected with a multihead cell-harvesting apparatus and the cpm value was measured.

$$\text{IL-1 activity} = \frac{\overline{\text{Sample cpm}} - \overline{\text{Control (Culture Fluid) cpm}}}{\overline{\text{Standard Sample cpm}} - \overline{\text{Control (Culture Fluid) cpm}}} \times \text{activity of the standard (ng/ml)}$$

The measurement of the IL-6:

The spleen cells were sampled in the asepsis condition. Then the samples were washed by Hank's liquor 2 times and nonserum

RPMI1640 liquor 1 time. Then the clear samples were adjusted to the 2×10^6/ml cell suspension with 5% FCS-RPMI liquor. 1 ml of the suspension was added to the round-bottom centrifuge tube. After adding the ConA (10ng/ml), the samples were cultured in 5% CO_2 at 37℃ for 72 hours.

The MH60 cells, which grew depending on the IL-6 and were on the logarithmic growth stage, were adjusted to the 1×10^5/ml cell suspension with the 5% FC-RPMI1640.

The 96-hole flat bottom cell culturing plate had added the MH60 cell suspension at the quantity of 100μl/hole and the culturing supernate 25μl/ hole. Then the fluid in each hole was adjusted to 200μl with the 5% FCS-RPMI 1640. Each sample was cultured with 3 copies and compared with the different solutions standard rHIL-6 and the pure culturing fluid. The plate was cultured in 5%CO_2 at 37℃ for 72 hours. 6 hours before the end of the culture, the samples were centrifuged. In each hole, the supernate 110μl was sucked out and the MTT 10μl was added. The samples were kept at 37℃ for 3 hours. And then the OD at the wavelength 570nm and 630nm were measured. The final OD value = OD 570nm – OD 630nm.

$$\text{IL-6 activity} = \frac{\text{SampleOD - Culturing Fluid ControlOD}}{\text{Standard SampleOD - Culturing Fluid ControlOD}} \times \text{Sample Dilution} \times \text{Activity Of The Standard (IU/ml)}$$

Table 5.2 The effect of Fengshiping on the IL-1, IL-6 ($\overline{X} \pm S$)

Group	Dose (g/kg)	Mouse number	IL-1 (ng/ml)	IL-6 (IU/ml)
Control	-	10	78.7±7.1	94.6±6.8

Fengshiping	7.5	10	59.3±4.9**	64.9±4.8**
	15	10	53.3±5.7**	60.5±4.3**
	30	10	54.4±4.8**	56.0±4.6**
	60	10	47.0±16.6**	56.6±6.1**
Tripterygium hypoglaucum (Levl.) Hutch.	5	10	57.6±4.7**	65.7±4.9**
cyclophosphane	0.02	9	44.5±7.7	49.6±6.7**

Based on the data in the table 5.2, the Fengshiping had an obvious inhibiting effect on the macrophage in producing of IL-1 and spleen cell in producing IL-6. Along with the increase of the dosage, the effect is enhanced.

5.3 The effect of Fenghsiping on the plasma NO in the AA rat

60 SD rats, 160～220g weight, half male and half female, were divided into 6 groups. The rats in the blank control group were injected the NS 0.5ml under the skin of the right postpede vola. Other rats were injected with the FCA 0.5ml at the same place as that of the control group. 18 days later, the AA model was built. Then the rats were given orally the corresponding medicines or the distilled water once a day for 5 days. 3 groups were given orally the solution of Fengshiping at a high, middle and low dilution. The positive group was given orally Glucosidorum Tripterygll Totorum. The blank control group and the model group were given orally the distilled water of the same volumn. 1 hour after the last administration, each rat's blood from the abdominal aorta was sampled at 2 ml. The plasma of the blood samples was separated and saved at - 70℃ for the measurement. The measurement of NO was done as per the directions of the NO reagent. 0.1ml plasma was added in 0.6ml reagent C and 0.4ml re-distilled water. After the mixture was shaken up, it was added in 0.1ml reagent D and cultured on the ice for 60 min. Then it was centrifuged at 12000 rpm for 2 min. The

supernate was separated. 0.6 ml supernate was mixed with 0.4ml re-distilled water and 0.1ml reagent A, and then it was cultured in the ice-water for 15 min. Then the mixture was added in reagent B 0.1ml and put at the room termperature for 1 hour. Then the new mixture OD was measured at the wavelength of 545nm. Based on the OD value of the sample, the content of NO was calculated on the standard curve. (See the result in table 5.3)

Table 5.3 The effect of Fengshiping on the plasma NO level in the AA rat ($\overline{X} \pm S$)

Group	Dose (g/kg)	Rat number	Content of NO (μmol/L)	y (y=Lgx)
Control	-	8	13.55±1.11*	1.131±0.032
AA model	-	9	17.56±4.15	1.235±0.097
Fengshiping	12	7	9.83±2.58**△△	0.985±0.087
Fengshiping	24	7	10.12±1.56**△△	1.001±0.067
Fengshiping	48	7	10.70±1.51**△△	1.026±0.062
Glucosidorum Tripterygll Totorum	0.006	7	15.25±3.48	1.173±0.099

Comparing to the model group*P<0.05 , **P<0.01 ; comparing to the Glucosidorum Tripterygll Totorum△△P<0.01

Based on the data in table 5.3, the NO level was higher in the model group than in the blank control group. The Fengshiping had an obvious effect on lowering the NO level in the AA rat. The Glucosidorum Tripterygll Totorum had the similar effect but its effect was weaker than that of the Fengshiping.

Experimental example 6 : The effect of Fengshiping on the T lymphocyte, CD_4, CD_8 and NK cells in the mouse.

6.1 The effect of Fengshiping on the transformation of lymphocytes in the normal mouse.

80 NIH mice, half male and half female, were divided into 8 groups randomly and given orally the corresponding medicines once a day for 10 days. 24 hours after the last administration, all the mice were killed to sample the spleen cells aseptically. Then the samples were washed with Hank's liquor 2 times and nonserum RPMI1640 liquor 1 time. Then the clear samples were adjusted to the 2×10^6/ml cell suspension with 5% FCS-RPMI liquor. The 96-hole flat bottom cell culturing plate was filled with the cell suspension at the quantity of 100μl/hole. Each sample was cultured with 3 copies. 2 holes were added with 2ng ConA each as the stimulating reagent. The other hole did not have any additions of the ConA and was kept as the control hole. The platter was cultured in 5% CO_2 at 37℃ for 72 hours. 14 hours before the end of the culture, each hole had 3H-TdR 0.1μCi added. The cells were harvested by the multihead cell harvesting instrument and had the cpm value measured. The average value was adopted as the sample's cpm value. The average value and the stimulating index of the different groups were compared directly. The stimulating index was calculated as following:

$$\text{Stimulating Index} = \frac{\text{Stimulated } \overline{\text{cpm}}}{\text{Control } \overline{\text{cpm}}}$$

See the result in tale 6.1

Table 6.1 The effect of Fengshiping on the lymphacy to transformation induced by ConA in the mouse ($\overline{X} \pm S$)

Group	Dose (g/kg)	Mouse number	cpm	Stimulating index
Control	-	10	20433±3579	25.87±3.06
Fengshiping	7.5	10	13566±1779**	27.29±7.67

	15	10	12708±1692**	18.04±3.76
	30	10	12809±2575**	16.17±4.37
	60	10	12090±1706**	19.05±3.80
Tripterygium hypoglaucum (Levl.) Hutch.	2.5	10	18038±3359	17.11±2.60
	5	10	12081±1039**	17.58±4.37
Cyclophosphane	0.02	9	9922±1145**	13.66±2.28

Comparing to the control group*P<0.05 , **P<0.01

According to the data in table 6.1, it indicated that the Fengshiping had an obvious inhibiting effect on the lymphocyte transformation and there was a dosage-effect relationship.

6.2 The effect of Fengshiping on the CD_4, CD_8 and NK cells

The experiment was same as 5.1. 24 hours after the last administration, the spleen cell samples were made into a 2×10^8/ml cell suspension with 5% FCS-RPIMl640. The quantity of CD_4, CD_8, NK cells and the rate CD_4/CD_8 were measured by the usural method.

The measurement of CD_4 and CD_8:

50μl of the spleen cell suspension was added on the glass to made the cell smear. The glass had been coated by the polylysine. The T cell of the mouse was set as the positive control sample. The cell smear was enveloped by the serum of the normal mouse after it was fixed by acetone. Then the enveloped sample was added with the antibody of CD_4 and CD_8 which were marked by the hominine biotin. It was incubated at 37℃ for 2 hours. Then the sample was added to the avidin labeled by the enzyme and held still for 10 min. After the substrate was added for 10 min, the mixed sample was washed and dyed with the hematoxylin for 2 min. Then the sample was dehydrated with the grade-alcohol and enveloped with gelatin-glycerol. 200 cells in the smear were chosen as the research target under the high power microscope.

$$\text{Content Of Cell} = \frac{\text{Dyed cell number}}{200} \times 100\%$$

The measurement of the NK cell:

The preparation of the EC cell: The spleen cells were sampled in the asepsis condition. Then the samples were washed by Hank's liquor 2 times and nonserum RPMI1640 liquor 1 time. Then the clear samples were adjusted to the 2×10^8/ml cell suspension with 5% FCS-RPMI liquor. This cell suspension was used as the EC.

The preparation of the TC cell: The Yack-1 cells, which were sensitive to the mouse NK cell and on the logarithmic growth phase, were adjusted to the 4×10^4/ml cell suspension. It was the TC.

Measurement: EC and TC, 100μl each were added in the 96-hole flat bottom cell culturing plate. Each sample was cultured with 3 copies and set 2 control samples: EC and TC. (EC control: EC100μl + 5% FCS RPMI 1640 100μl ; TC control : TC100μl + 5% FCS RPMI 1640 100μl). The samples were cultured in 5% CO_2 at 37℃ for 24 hours. 6 hours before the end of the culturing, the samples were centrifuged and 110μl supernate were sucked out of each hole. And then 10μl of the MTT were added to the holes. After setting at 37℃ for 3 hours , the mixed samples OD values were measured at the wavelengths of 570nm and 630 nm. The OD of each hole=OD570nm - OD630nm.

$$\text{Activity Of NK} = \left(1 - \frac{\text{Sample } \overline{OD} - \text{EC Control } \overline{OD}}{\text{TC Control } \overline{OD}}\right) \times 100\%$$

Table 6.2 The effect of Fengshiping on the CD4, CD8, NK cell ($\overline{X} \pm S$)

Group	Dose (g/kg)	Mouse number	CD4 (%)	CD8 (%)	CD4/CD8	NK
Control	-	10	20.80±2.94	14.80±2.49	1.42±0.18	40.13±4.89
Fengshiping	12	10	19.14±2.91	13.43±2.51	1.43±0.08	31.94±4.52**$^{\Delta\Delta}$
	24	10	17.30±2.51**	12.00±2.40	1.46±0.16	35.36±3.40*$^{\Delta\Delta}$
	36	10	16.30±2.50**	11.23±2.94**	1.49±0.20	31.06±3.53**$^{\Delta\Delta}$
Tripterygium hypoglaucum (Levl.) Hutch.	8	10	16.25±2.25**	11.50±2.45	1.44±0.18	32.20±2.00**
Cyclophosphane	0.02	10	11.50±2.50**	4.10±1.20**	2.91±0.53**	23.10±3.66**

Comparing to the control group*P<0.05 , **P<0.01 ; comparing to the cyclophosphane$^{\Delta\Delta}$P<0.01

According to the table 6.2, Fengshiping has some inhibiting effect on CD_4 and CD_8, there was a relation between the dosage and the effect, but the dosage-effect curve was smooth. The effective dosage of Fengshiping on the inhibiting of CD_4 was 24g/kg. The minimum effective dosage on inhibiting the CD_8 was 36g/kg. Fengshiping had no obvious effect on the rate of CD_4/CD_8. Cyclophosphane had an obvious effect on the inhibiting of both kind of cells, and the inhibiting effect on the CD_8 was very powerful, which could increase the rate of CD_4/CD_8 magnificently.

As for NK cell, the Fengshiping had a remarkable inhibiting effect, but the dosage-effect relationship was not certain. At the same time, the cyclophosphane had shown an obvious inhibiting effect on the NK cell. On the dosage of 20mg/kg, the inhibitiong effect of cyclophosphane was significantly different from that of the Fengshiping at the 3 dosages: 12, 24 and 36g / kg.

6.3 The effect on the transformation and function of the T lymphacyto in the AA mouse.

NIH mice, 20±2g weight, were injected with 0.05 ml FCA under the skin of the right postpede vola to build the AA model. The mice in the control group were injected 0.05ml NS at the same place. 3 weeks later, after the AA model was built, all the mice were given orally the corresponding medicines once a day for 5 days. 5 days later, all the mice were sampled, and the blood was used to make a blood smear. The smears were dyed by the esterase. Then the smears were observed under an oil immersion lens to calculate the percent of the positive-dyed cells (it represented the content of the T cells in the blood). The spleen cells of

the mice were sampled while under anaesthesia and then the cell samples were prepared in a single cell suspension. The cell suspension was washed by PBS and then its supernate was abandoned. The rest had blood cytolysate 4ml added. The mixed sample was shaked for 2 ~ 3 min to solute the RBC. After the RBCs were destroyed, the sample was centrifuged to separate and abandon the supernate. The sample without supernate was washed by the luminescence lotion for 2 times. Then it was centrifuged to separate and abandon the supernate. In the next step, the sample was adjusted to the 1×10^6/ml cell suspension. Each tube was added with 50μl diluted antibody of CD_4 and CD_8 . Then the tubes were cultured at 4℃ for 1 hour. After the culture, the samples were washed with the luminescence solution 2 times and 2ml of the fixing fluid was added. After fixing, the samples were filtered through the 400-mesh screen to the FCA tube. The filtered samples were analyzed by the flow cytometer (FCM). The result is shown in the table 6.3.

Table 6.3 The effect of Fengshiping on the T cell in the AA mouse

$(\overline{X} \pm S)$

Group	Dose (g/kg)	ANAE+ (%)	CD4+ (%)	CD8 (%)	CD4+/CD8+
Control	-	50.60±4.25	26.13±1.16	15.56±0.68	1.68±0.03
AAmodel	-	49.00±4.22▲	32.56±2.87**	13.59±1.03**	2.49±0.16**
Fengshiping	7.5	49.13±4.03▲	27.30±1.76##▲	15.98±1.11##▲	1.71±0.04##▲
	15	49.31±3.29▲	27.96±1.67##▲	16.23±1.27##▲	1.73±0.05##▲
	30	48.56±3.23▲	26.75±1.94##▲	15.58±1.29##▲	1.72±0.04##▲
Glucosidorum Tripterygll Totorum	0.012	48.88±2.89▲	27.88±1.99##▲	16.33±1.31##▲	1.70±0.03##▲

n=8 , comparing with the control group*P<0.05 , **P<0.01 ; comparing with the model group# P<0.05 , ## P<0.01 ; comparing with the control

group▲P>0.05

According to the data in table 6.3, there was no significant difference in the different groups on the ANAE positive cell. But in the AA mouse, the increase of the CD_4 was significant, while the decrease of CD_8 was significant too. So the rate of CD_4/CD_8 had a remarkable increase. The result indicated that the Fengshiping could adjust the CD_4, CD_8 and CD_4/CD_8 to the normal range.

Experimental example 7: The effect of Fengshiping on the phagocytic function of the macrophage in the mouse abdominal carvity.

50 NIH mice, 18~ 22g weight, half male and half female, were divided into 5 groups and given orally the corresponding medicine solutions at the same volume. The administration was once a day for 7 days. 1 hour after the last administration, all the mice were injected with 0.2ml 10 % chick RBC into the abdominal carvity. 4 hours later, all the mice were killed and the fluid in the abdomincal cavity was sampled. The liquor samples were dropped on the glass and the number of macrophages were counted which had phagocytized the CRBC and the number of the CRBC in one macrophage were also counted. (See the result in table 7)

Table 7 The effect of Fengshiping on the CRBC phagocytosis function of the macrophage in ICR mouse abdominal cavity ($\overline{X} \pm S$)

group	dose (g/kg)	Mouse number	Percent of phagocytosis (%)	phagocytosis index
Control	-	10	25.75±9.40	1.28±0.20
Fengshiping	27	10	33.20±12.77	1.46±0.36
Fengshiping	40.5	10	35.20±10.16	1.21±0.20
Fengshiping	60.9	10	37.78±20.14	1.53±0.32

dexamethasone	0.005	10	8.33±10.13*	1.10±0.18

*P<0.05

According to the table 7, the Fengshiping had no obvious effect on the phagocytosis function of the macrophage in the mouse abdominal cavity.

Experimental example 8: The effect of Fengshiping on the hyperfunction of the capillary permeability in the mouse abdominal cavity.

90 NIH mice, 18~22g weight, half male and half female, were divided into 9 groups and given orally the corresponding medicine solutions of the same volume. The medicines were given once a day for 3 days or just 1 time. 1 hour after the last administration, each mouse was injected with 0.7% HAC – NS solution into the abdominal cavity. At the same time, each mouse was injected with the 0.5% Evans blue – NS solution into the vessel at the dose of 0.1ml/10 g. 30 min later; all the mice were killed by cervical disjoint. The abdominal cavity was opened and washed with the 5ml NS. The NS used was collected and adjusted to 8ml by the pure NS as the sample. The samples were centrifuged at 3000 rpm to get the supernate. The supernate OD was measured at the wavelength of 590nm. (See the result in table 8)

Table 8 The effect of Fengshiping on the hyperfunction of the capillary permeability induced by the acetic acid in the mouse abdominal cavity ($\overline{X} \pm S$)

Group	Dose (g/kg)	Administration	Mouse number	Leakage of the tincture (OD)	P value
Control	-	-	10	0.29±0.13	
Fengshiping	27	qd×1	10	0.26±0.14	>0.05

Fengshiping	40	qd×1	10	0.25±0.10	>0.05
Fengshiping	60	qd×1	10	0.25±0.09	>0.05
Control	-	-	10	0.28±0.15	
Fengshiping	27	qd×3	10	0.25±0.12	>0.05
Fengshiping	40	qd×3	10	0.18±0.10	<0.05
Fengshiping	60	qd×3	10	0.15±0.13	<0.05
dexamethasone	0.15	qd×3	10	0.11±0.07	<0.01

According to the data in table 8, it indicated that Fengshiping could obviously inhibit the hyperfunction of the capillary permeability induced by the acetic acid in the mouse abdominal cavity if it was given the medicine for 3 days continuously. If the medicine was given for just 1 time, the inhibiting effect was not obvious.

Experimental example 9: The effect of Fengshiping on the pleuritis exudation and the inflammatory cell aggregation induced by the carrageenan.

The mice were divided into 5 groups at random and injected with 0.5% Evans blue NS solution into the caudal vein at the dosage of 0.1ml/10g. Then the mice were injected with the 0.03ml 1% carrageenan in the right chest cavity with the special syring needle. 4 hours and 32 hours after the injection, the corresponding mice were killed and had their abdominal cavity opened to expose the diaphragm. 2ml of the solution were injected to the chest cavity 2 times with a 1 ml injector. The solution was collected and saved in a test tube. 20μl of the solution collected was added into the 400μl WBC dilution. The WBC in the mixed dilution was counted under the microscope. The rest of the solution was centrifuged at 3000rpm for 10 min. The supernate of the solution's OD was measured at the wavelength of 600nm. The OD value of the sample should be corrected with the correspondent OD value of

the pure solution. (See the result in table 9)

Table 9 The effect of Fengshiping on the inflammatory cell aggregation induced by the carrageenan ($\overline{X} \pm S$)

Group	Dose (g/kg)	WBC number(2×105)		Tincture exudation (OD)	
		4h	32h	4h	32h
Control	-	46.0±6.9	16.0±9.6	0.156±0.066	0.109±0.019
Fengshiping	27	26.8±4.5*	14.2±8.0	0.121±0.062	0.116±0.031
Fengshiping	40.5	10.9±4.0**	17.3±4.6	0.100±0.048	0.153±0.032
Fengshiping	60	8.0±5.5**	6.6±4.7*	0.129±0.066	0.092±0.051
dexamethasone	0.05	12.7±10.2**	4.4±4.0*	0.085±0.045	0.063±0.017

*P<0.05 , **P<0.01

According to the table 9, it indicated that the Fengshiping had an obvious inhibiting effect on the inflammatory cell aggregation. The effect was powerful at the early stage. The regression equation on the data of the fourth hour was as follows: y=44.13 - 2.01x , r= - 0.9625. The effect on the late stage was weak. At the high dosage of 20g/kg, the medicine could affect the aggregation of the WBC. But it had no obvious effect on the pleuritis exudation.

Experimental example 10: Effect on aggregation of leucocyte in rats' CMC sac.

Sixty four SD rats, 150-180g weight, half male and half female, were randomly divided into 8 groups, which were given orally the same

volume and different dosage of drug liquid once a day, lasting 3 days. A day before experiment, rats were injected with 20ml 1% CMC solution into the sac at the rat's back caused by 20ml air injection before the experiment. 3.5 hours and 7.5 hours later, 0.1ml of liquid in the sac was extracted each time, and was colored in 0.01% brilliant cresyl blue solution and leucocytes were counted in the sac liquor under a microscope. The results are shown in the table 10.

Table 10 effect on leucocyte counts of carboxymethyl cellulose sac of rats with Fengshiping ($\overline{X} \pm S$)

groups	dosage (g/kg)	rats number	WBC count(×107/L)	
			3.5 hrs	7.5 hrs
control	-	8	9.7±4.2	57.7±17.3
Fengshiping	27×1	8	8.5±3.5	39.4±16.5
Fengshiping	40×1	8	8.7±7.3	35.3±23.2
Fengshiping	60×1	8	6.6±3.3	18.1±8.6**
Control	-	8	10.97±6.7	35.6±11.2
Fengshiping	27×3	8	15.4±9.7	38.6±15.5
Fengshiping	40×3	8	4.8±3.4**	18.4±12.2**
Fengshiping	60×3	8	3.0±2.8**	11.0±9.2*
cortisone	0.1×3	8	14.2±8.0	41.7±16.0
Control	-	8	10.9±3.0	41.3±6.9
Fengshiping	18×7	8	6.2±3.0*	11.4±6.4*
Fengshiping	27×7	8	3.7±1.7**	6.4±3.1**
Fengshiping	40×7	8	2.5±1.9**	5.9±3.9**
cortisone	2mg×1	8	1.5±0.7**	3.0±1.0**

Compared with control group**P<0.01

According to table 10, the Fengshiping could inhibit significantly, aggregation of leucocyte in the rats' CMC sac, and the inhibition showed

apparent dosage-effect correlation, which was stronger as administration time lasted. With administration continuing seven days, wandering of leucocyte could be inhibited significantly at dosage of 18g/kg, at the same time , there was also very strong inhibition with cortisone injection into the sac.

Experimental example 11: The effect on croton oil-induced swelling in the ears of mice.

60 NIH mice with weight of 18 ~ 22g, male and female accounting for half and half, were divided into 6 groups, which were given orally with the same volume and different dosage of drug liquid or tragacanth liquid, once a day, lasting 3 days. 1 hour after the final administration, 2% croton oil mixure of 0.02ml was embrocated uniformly on both sides of the left ears of the mice, and after 4 hours, the mice were put to death by snapping their cervical vertebra. The left and right ears were cut down, then inflammatory and control ears were weighed by certain means. Differences of weight between left and right ears was the swelling extent of the ears, with results shown in table 11.

Table 11 Effect on croton oil-induced swelling of the ears of mice with Fengshiping ($\overline{X} \pm S$)

Groups	dosage (g/kg)	rats number	Degree of ears' swelling (mg)	inhibition rate (%)	P value
Control group	-	10	44.38±9.40		
Fengshiping	27	10	39.05±12.33	12.00	>0.05
Fengshiping	40	10	36.65±5.83	17.64	<0.05
Fengshiping	60	10	34.91±9.71	21.34	<0.05
dexamethasone	0.003	10	14.13±5.75	68.16	<0.01

As seen from table 11, that Fengshiping had remarkable inhibition to croton oil-induced swelling of the ears of mice, and had dosage-effect

correlation, but the curve was even and smooth. There was significant inhibition effect at 13.5g/kg of dosage.

Experimental example 12: Effect on acetic acid-induced twisting reaction of mice.

60 Kuming mice with weight of 18 ~ 22g, male and female accounting for half and half, were randomly divided into 6 groups, which were given orally with different dosages of drug liquid or tragacanth solution. 1 hour after administration, 0.7% HAC saline of 0.2ml was injected, sc, and the mice were placed in an aquarium and oberved the latent period before the twisting reaction of each mouse and the twisting times in 20 minutes, with the results shown in table 12:

Table 12 The effect of Fengshiping on acetic acid-induced twisting reaction of mice ($\overline{X} \pm S$)

groups	dosage (g/kg)	Rats numbers	Twisting times	Latent time (minute)
Control	-	10	34.6±14.1	3.13±0.80
Fengshiping	27	10	28.2±5.76	3.82±0.85
Fengshiping	40	10	31.0±18.4	3.86±2.00
Fengshiping	60	10	20.7±12.3*	3.95±1.42
Tripterygium hypoglaucum (Levl.) Hutch.	20	10	25.1±11.9	3.60±0.93
morphine hydrochloride	10mg/kg	10	0.0±0.0	0.00±0.00

It was seen from table 12 that large doses of Fengshiping could delay the latent time before the HAC-induced twisting reaction and significantly reduce the twisting times in 20 minutes, which indicated Fengshiping had the effect of aberration in some degree.

Experimental example 13: Effect on hemorheology of AA rats.

Each of SD rats, 180±20g weight, were injected intracutaneously

with 0.05ml Freund's complete adjuvant on the right back foot metatarsal, and developed into adjuvant arthritis models. Each of the rats of negtive control group were injected intracutaneously with 0.05ml salin on the right back foot metatarsal. Three weeks after models were built, the rats were divided into model group, large, middle, small dosage groups, negtive control group and positive control group which was administered with Glucosidorum Tripterygll Totorum. The rats were given the medicines orally once a day, lasting 5 days. 1 hour after administration for the last time, 3ml of blood was taken from abdominal aorta of rats and placed into test tube with 1% heparin as decoagulant, in which the whole blood viscosity was measured at shear rate of 230, 115, 46, 23, 11.5, $5.75S^{-1}$ with an NXE-1 cone and plate viscometer. The plasma viscosity was measured with a WTP-BII adjustable constant pressure capillary viscosimeter. The haematocrit, erythrocyte aggregation index was measured with the centrifugation method of packed cell volume. The rigidity index was calculated from the above-mentioned data. All the results are shown in table 13.

Table 13 Effect on hemorheology of adjuvant arthritis model rats ($\overline{X} \pm S$)

Groups	Control group	Model group	Fengshiping (30g/kg)	Fengshiping (15g/kg)	Fengshiping (7.5g/kg)	Glucosidorum Tripterygll Totorum (6mg/kg)
whole blood viscosity (mPa.s)						
230S-1	4.43±0.09	4.92±0.15**	4.56±0.09##	4.49±0.11##	4.54±0.16##	4.66±0.28#
115S-1	5.17±0.25	5.81±0.19**	5.33±0.09##	5.32±0.10##	5.16±0.14##	5.60±0.48#
46S-1	6.84±0.11	7.20±0.18**	6.56±0.13##	6.59±0.09##	6.67±0.14##	6.70±0.48#
23S-1	8.10±0.15	8.23±0.38	7.95±0.22	7.93±0.12	7.97±0.14	8.02±0.14
11.5S-1	9.35±0.08	9.78±0.10**	9.40±0.08##	9.45±0.10##	9.30±0.133	9.31±0.12##
6.5S-1	11.03±0.14	12.66±0.31**	11.21±0.21##	11.29±0.19##	11.60±0.40##	11.42±0.52##
Plasma viscosity (mPa.s)	1.158±0.032	1.248±0.040**	1.161±0.011##	1.154±0.023##	1.156±0.018##	1.158±0.029##
corpuscular volume (%)	46.13±2.31	41.33±1.12**	45.10±2.39##	44.33±1.52##	45.71±1.04##	46.03±3.59##
erythrocyte aggregation index	2.49±0.032	2.58±0.083*	2.46±0.066#	2.49±0.094#	2.44±0.048##	2.45±0.091#
rigidity index	6.155±0.536	7.127±0.557**	6.506±0.558	6.525±0.146	6.394±0.200#	6.621±0.883

Compared with negative control group*P<0.05 , **P<0.01 ; compared with model control group# P<0.05 , ## P<0.01

According to the table 13, the hemorheology of AA rats was changed significantly. The whole blood and plasma viscosity increased, haematocrit decreased, aggregation index and rigidity index of erythrocyte increased. The Fengshiping could significantly improve the above-mentioned indexes of hemorheology.

Pharmacological effects of Fengshiping have been proved by the above-mentioned experiments. Many important pharmacological effects of Fengshiping had favorable dosage-effect correlation, which implied the best therapeutic effectiveness might be obtained by adjusting the drug dosage at clinic.

The clinical studies on Fengshiping were carried on in China, Japan and Austrilia. Theses studies were operated according to international criterion related disease classification about diagnosis, therapy and curative effect. The effective rate for RA was around 94%, and its remarkable effective rate was around 60%. It could improve the symptoms such as morning stiffness, swelling and pain and so on and the related items. The results showed in table 14 ~ 21.

Table 14 Compared effect of treatment group with control group

Groups	Cases	remission(clinical recovery)	Notable effect	Effective	No effect	Notable effect rate (%)	Effective rate (%)
Treatment group	32	5	14	11	2	59.38	93.74
Control group	30	3	10	12	5	43.33	83.33

Table 15 Influence of IgG, IgA and IgM ($\overline{X} \pm S$)

Groups	cases	IgG		IgA		IgM	
		pre -	post -	pre -	post -	pre -	post -
Normal	32	12.45±1.48		2.37±1.00		1.58±0.59	
Treatment group	32	16.92±3.49	14.17±1.39**	3.65±1.03	2.39±1.18**	1.89±0.88	1.48±1.01
Control	30	17.03±4.12	15.14±2.21**	3.45±1.86	2.32±1.75**	2.03±0.95	1.76±1.28

Comparing with pre-treatment **P<0.01

Table 16 Influence of C3 and C4($\overline{X} \pm S$)

groups	cases	C3		C4	
		pre -	post -	pre -	post -
normal group	32	0.62±0.13		0.14±0.15	
Treatment group	32	1.88±0.72	1.25±0.66**	0.48±0.12	0.26±0.06*
Control group	30	2.13±0.64	1.56±0.62**	0.40±0.16	0.25±0.07**

Comparing with before therapy *P<0.05, **P<0.01

Table 17 Influence of ESR and CRP ($\overline{X} \pm S$)

Groups	cases	ESR		CRP	
		pre-	post-	pre-	post-
Normal	32	8.37±5.26		4.12±1.88	
Treatment	32	66.58±9.01	30.31±6.53**	13.35±6.67	8.86±3.34*
control	30	73.33±9.09	35.83±11.61**	14.21±6.29	9.04±3.15**

Comparing with pre-treatment *P<0.05, **P<0.01

Table 18 Compared with power of gripping pre- and post-treatment ($\overline{X} \pm S$)

groups	Treatment Group		Control Group	
	pre -	post -	pre -	post -
Gripping power of left hands (mmHg)	39.13±20.24(15)	80.47±34.61**(15)	24.00±17.63(21)	55.15±23.27**(21)
Right hands	35.85±22.46(15)	85.32±36.32**(15)	22.80±12.32(21)	58.17±20.59**(21)

Comparing with pre-treatment *P<0.05, **P<0.01

Table 19 Influence of arthrosis swelling and pain and morning stiffness time ($\overline{X} \pm S$)

Items	Treatment Group		Control Group	
	pre -	post -	pre -	post -
arthrosis swelling and pain	5.79±0.52	3.14±0.83*	5.56±2.15	3.92±0.26*
morning stiffness time (minute)	50.33±6.47	20.24±3.27**	48.75±8.34	27.50±3.78**

Comparing with pre-treatment *P<0.05,**P<0.01

Table 20 Influence of RF changing to negative

Groups	Cases	RF negative		
		Pre - treatment	Post - treatment	Rate of negative turnaround (%)
Treatment group	32	24	11	54.2
Control group	30	18	10	44.4

Not only did it have significant effects, but also Fengshiping can make items such as SIL-2R, STNF, SIL-6R in plasma decrease, results showing in the Table 21.

Table 21 inflence of main indes such as SIL - 2R, STNF αnd SIL - 6R ($\overline{X} \pm S$)

groups	Cases	SIL - 2R(u/ml)		STNF R1(ng/ml)		SIL - 6R(ng/ml)	
		pre -	post -	pre -	post -	pre -	post -
Normal	32	299±68 (n=32)		1.56±0.48 (n=24)		72.05±18.26 (n=22)	
Fengshiping	15	683±189	381±157**	2.87±0.66	1.75±0.54**	136.18±28.57	90.15±20.12**
Control	10	765±203	412±167**	2.63±0.72	2.38±0.39 (n=8)	148.21±30.31	99.02±26.70**

Comparing with pre-treatment **P<0.01

It was proved that the above-mentioned results of the invention could be realized by the ways as follows.

Example of exploitation1:

Epimedium brevicornum Maxim. 2222g

Tripterygium hypoglaucum (Levl.) Hutch. 2222g

Lycium barbarum L. 1111g

Cuscuta chinensis Lam. 1111g

Tripterygium hypoglaucum (Levl.) Hutch. was cut into pieces, extracted for three times with 13, 10, 10-times water , each time lasting 1 hour; *Epimedium brevicornum* Maxim was cut into segments, extracted three times with 15, 10, 10-times water, each time lasting 1 hour; *Lycium barbarum* L. was crushed into coarse powder, and immersed in 20-times water of 80℃ for 1 hour; *Cuscuta chinensis* Lam. was crushed into coarse powder, immersed in 31-times water of 80℃ for 1 hour; the decoction fluid or immersion fluid of four herbs were filtrated respectively, poured across macropore polymeric adsorbent resin columns, and eluted with 70% alcohol. When the color of effluent became deep significantly, eluent started to be collected; when the color of effluent became very weak, elution collection was ended. The alcohol in the eluent of each herb was recovered. Then the fluid without alcohol was concentrated, dried to get the finally extract powder; officinal starch was blended with the four kinds of extract powder to 200g, mixed up uniformly and encapsuled into 1000 capsules. Each capsule which was prepared with the invented method thereof, was composed of 0.2g drug extract and contained at least 2.0mg of icariin $C_{33}H_{40}O_{15}$. The regular dosage is: oral administration, three times every day, three capsules for each time.

Example of exploitation 2:

Tripterygium hypoglaucum (Levl.) Hutch.2000g

Epimedium brevicornum Maxim.2000g

Tripterygium hypoglaucum (Levl.) Hutch. was cut into pieces, extracted three times with 13, 10, 10-times water, each time lasting 1 hour; *Epimedium brevicornum* Maxim. was cut into segments, extracted three times with 15, 10, 10-times water, each time lasting 1 hour; decoction fluid of herbs were filtrated respectively, poured across macropore polymeric adsorbent resin column, eluted with 70% ethanol, when the color of effluent became deep significantly, eluent was commenced to collect; when the color of effluent became very weak, elution was over. The alcohol in the eluent of each herb was recovered. Then the fluid without alcohol was concentrated, dried to get the finally extract powder; officinal starch was blended with the extractive drug powder, and mixed up uniformly, loaded to 1000 capsules. Each capsule which was prepared with the inventive method thereof, is composed of 0.2g drugs extractive, contains at least 2.0mg of icariin $C_{33}H_{40}O_{15}$. regular dosage is: oral administration, three times every day, three capsules for each time.

Example of exploitation 3:

Tripterygium hypoglaucum (Levl.) Hutch.2000g

Epimedium brevicornum Maxim.2000g

Lycium barbarum L. 1000g

Tripterygium hypoglaucum (Levl.) Hutch. was cut into pieces, extracted three times with 13, 10, 10-times water, each time lasting 1 hour; *Epimedium brevicornum* Maxim.was cut into segments, extracted three times with 15, 10, 10-times water, each time lasting 1 hour; *Lycium*

barbarum L. was crushed to coarse powder, and immersed in 20-times water of 80℃ for 1 hour; decoction fluid or immersion fluid of four herbs were filtrated respectively, poured across a macropore polymeric adsorbent column, eluted with 70% alcohol, when the color of effluent became deep significantly, eluent was started to be collected; when the color of effluent became very weak, elution was over. The alcohol in the eluent of each herb was recovered. Then the fluid without alcohol was concentrated, dried to get the finally extract powder; officinal starch was blended with the extractive drug powder, and mixed up uniformly, loaded to 1000 capsules. Each capsule which was prepared with the inventive method thereof, is composed of 0.2g drugs extractive, contains at least 2.0mg of icariine $C_{33}H_{40}O_{15}$. Regular dosage is: oral administration, three times every day, three capsules for each time.

Example of exploitation 4

Tripterygium hypoglaucum (Levl.) Hutch.2000g

Epimedium brevicornum Maxim.2000g

Cuscuta chinensis Lam. 1000g

Tripterygium hypoglaucum (Levl.) Hutch. was cut into pieces, extracted three times with 13, 10, 10-times water , each time lasting 1 hour; *Epimedium brevicornum* Maxim.was cut into segments, extracted three times with 15, 10, 10-times water, each time lasting 1 hour; *Cuscuta chinensis* Lam. was crushed to coarse powder, immersed in 31-times water of 80℃ for 1 hour; decoction fluid or immersion fluid of the herbs were filtrated respectively, poured across a macropore polymeric adsorbent column, eluted with 70% ethanol, when the color of effluent became deep significantly, collection of eluent began; when the color of effluent became very weak, elution was over. The alcohol in the

eluent of each herb was recovered. Then the fluid without alcohol was concentrated, dried to get the finally extract powder; officinal starch was blended with extract drug powder, and mixed up uniformly, loaded to 1000 capsules. Each capsule which was prepared with the inventive method thereof, is composed of 0.2g drug extract, contains at least 2.0mg of icariin $C_{33}H_{40}O_{15}$. Regular dosage is: oral administration, three times every day, three capsules for each time.

Example of exploitation 5

Tripterygium hypoglaucum (Levl.) Hutch. 2000g

Cuscuta chinensis Lam. 1000g

Tripterygium hypoglaucum (Levl.) Hutch. was cut into pieces, extracted three times with 13, 10, 10-times water, each time lasting 1 hour; *Cuscuta chinensis* Lam. was crushed to coarse powder, immersed in 31-times water of 80℃ for 1 hour; decoction fluid or immersion fluid of the herbs were filtrated respectively, poured across the macropore polymeric adsorbent column, eluted with 70% ethanol, when the color of effluent became deep significantly, collection of the eluent began; when the color of effluent became very weak, elution was over. The alcohol in the eluent of each herb was recovered. Then the fluid without alcohol was concentrated, dried to get the finally extract powder; officinal starch was blended with extractive drug powder, and mixed up uniformly, and loaded to 1000 capsules. The dose of capsules administered every day, which was prepared with the inventive method thereof, was equivalent to 30g of crude drugs.

Example of exploitation 6:

Tripterygium hypoglaucum (Levl.) Hutch.2000g

Lycium barbarum L. 1000g

Tripterygium hypoglaucum (Levl.) Hutch. was cut into pieces, extracted three times with 13, 10, 10-times water , each time lasting 1 hour; *Lycium barbarum* L. was crushed to coarse powder, and immersed in 20-times water of 80℃ for 1 hour; decoction fluid or immersion fluid of the herbs were filtrated respectively, poured across macropore polymeric adsorbent column, eluted with 70% ethanol, when the color of effluent became deep significantly, collection of the eluent began; when the color of effluent became very weak, elution was over. The alcohol in the eluent of each herb was recovered. Then the fluid without alcohol was concentrated, dried to get the finally extract powder; officinal starch was blended with extractive drug powder, and mixed up uniformly, and loaded to 1000 capsules. The dose of capsules administered every day, which was prepared with the inventive method thereof, was equivalent to 30g of crude drugs.